Pedobiologia **21**, 341—345 (1981)

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# Esterases in *Folsomia candida* (Collembola: Isotomidae) Changes in isozyme titer during the molt cycle

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With 2 figures

(Accepted: 12. 03. 80)

## 1. Introduction

Much attention has been focussed on the use of esterase electrophoretic patterns in insects and related groups. Tissue specific esterase isozyme patterns and developmental changes in isozyme composition or titer have been reported (COOK & FORGASH 1965; CLEMENTS 1967, BECKENDORF & STEPHEN 1970; and AHMAD 1976). Esterase variation has been used to estimate genetic variation within and between populations (WAGNER & SELANDER 1974) and as a tool in systematics, especially within the Lepidoptera (HUDSON & JUI 1976; SELL et al. 1974).

Recently, esterases were examined in several Collembolan species (HART & ALLAMONG, 1979) to help answer evolutionary and taxonomic questions in the Collembola. In each species examined, esterase variation was encountered and quantified. One of the species examined in the study was *Folsomia candida* (WILLEM), a parthenogenic soil Collembola.

ASHER & SNIDER (1975) recovered two esterase electrophoretic variants from laboratory populations of *F. candida*. The variants differed in the relative position of the fastest migrating anodal isozyme band. The two esterase patterns were heritable and were labelled the "fast" and "slow" types. Both genetic variants also exhibited variation correlated with the molt cycle (physiological variation): the two fastest anodal migrating bands disappeared some time previous to molting and returned after exuviation was completed.

The present study characterizes the changes in esterase patterns during the molt cycle of *F. candida*, in greater detail than previous studies, so that the variation involved in the molt cycle can be removed from variation involved in species identification. Emphasis is placed on changes occurring during Phase 2 (recognizable by lack of gut contents, PALÉVODY & GRIMAL 1976). This phase was chosen because egglaying in Phase 5 could be observed, assuring uniform staging of the *F. candida* individuals.

## 2. Material and Methods

The *F. candida* used in this study originated from stock cultures maintained in the laboratory for over fifteen years. The culture methods employed for stocks and isolated females were those of SNIDER (1973), using clear plastic jars (2.5 × 3.5 cm) with snap-on lids, filled to a depth of .5 cm with a 1:1 plaster-charcoal mixture. The charcoal was Darco G60, from Sargent-Welch Scientific Company, Skokie, Illinois, USA. Distilled water was added periodically to maintain humidity close to 100%. All animals were maintained at a constant temperature of 15.5 °C, in darkness except for observation periods. Powdered yeast was used as the food source.

Adult *F. candida* have an instar duration of 6—9 days when raised at 15.5 °C (SNIDER & BUTCHER 1973), with alternating egglaying and non-egglaying instars. PALÉVODY & GRIMAL (1976) divide the events occurring during the molt cycle into five repeating phases:

| | | Description | Duration |
|---|---|---|---|
| Phase 1 | Intermolt 1 | (Feeding Period 1) | 4—5 days |
| Phase 2 | Fasting 1 | (Gut Empty) | 48—54 hours |
| ---M1---------- | Exuviation 1 | ---------- | ---------- |
| Phase 3 | Intermolt 2 | (Feeding Period 2) | 5 days |
| Phase 4 | Fasting 2 | (Gut Empty) | 48—54 hours |
| ---M2---------- | Exuviation 2 | ---------- | ---------- |
| Phase 5 | Egglaying | (Partial Feeding) | 24 hours |
| ---------- | New Phase 1 | --------(etc.) | ---------- |

Their terminology will be followed in this paper.

Equal-sized *F. candida* were isolated from pure stocks, each stock containing animals of either the fast migrating (F) or the slow migrating (S) esterase type. The newly isolated females were observed daily for two weeks to assure that their instar duration, feeding habits and phase of egglaying were within the normal biological range for *F. candida* (SNIDER 1973); these observations served to establish the molt cycle phase.

After two weeks of daily observation, the animals were observed every six hours for entry into Phase 2 (the first 6 hour interval in which gut contents were absent). Phase 2, which lasts about two days, was divided into six hour intervals, (0—6 hours into Phase 2, 6—12 hrs, 12—18 hrs, 18—24 hrs, 24—30 hrs, 30—36 hrs, 36—42 hrs, 42—48 hrs and 48—54 hrs into Phase 2). *F. candida* were killed by freezing and stored at —20 °C as they entered these intervals until 32 individuals had been collected for each interval in Phase 2. This procedure was followed for both fast and slow genetic variants. There was no detectable difference in esterase activity between fresh *F. candida* and those frozen at —20 °C for up to six months.

Starch gel electrophoresis using an assay constructed from BREWER (1970) resulted in a zymogram of six clear esterase bands. Starch gels (Electrostarch Company, Madison, Wisconsin, USA) were made with a gel buffer of .005 M Histidine, *p*H 8.9. Whole body homogenates of individual *F. candida* crushed in gel buffer were used as the enzyme source. Tray buffers consisted of .41 M Sodium citrate, *p*H 8.0 and 10% NaCl (in the electrode chamber). After electrophoresis (5 hrs, 4 °C, 5 mA), the gel was incubated in a staining mixture containing 1 mg/ml Fast Blue RR in .04 M Trizma base, *p*H 6.25. The substrate, alpha naphthylacetate, was dissolved in acetone and added to the staining mixture. The final concentration of substrate was .2 mg/ml while acetone final concentration was 2% (v/v).

The stained gel slices were examined and scored for the presence of the two fastest migrating esterase bands. Any detectable esterase activity was scored as "enzyme present"; no quantification of enzyme levels was attempted. Control *F. candida* homogenates, staged to a time period of two days into Phase 3 (when all enzyme bands were present), were electrophoresed simultaneously to verify the location of all six esterase bands.

## 3. Results

Starch zymograms of control animals (2 days into Phase 3) of both esterase genetic variants show six bands of activity (Fig. 1a, b). There was no detectable change in activity in bands 1—4 through the molt cycle, but the fifth and sixth bands disappeared and reappeared during Phase 2 (Fig. 1c, d, e, f).

Division of Phase 2 into shorter time periods showed that band 6 was the first to disappear with minimal levels in the population occurring 6—12 hours into Phase 2 (Fig. 2). Band 6 then rapidly reappeared in the population so that by 42—48 hrs into Phase 2, 100% of the assayed population possessed detectable levels of the enzyme. Band 5 disappeared from the population at a later time, reaching minimal levels in the population by 12—18 hrs into Phase 2. Minimum levels of band 5 were maintained for approximately 18 hrs. Recovery of band 5 activity did not begin until 36—42 hrs into Phase 2, and enzyme activity had not returned to 100% of the population even after 48—54 hours into Phase 2.

There appeared to be no substantial differences in the cycling of the esterase bands between the fast and slow genetic variants, but the data suggest that the "slow" genetic variant may complete the cycle of loss and gain of both bands 5 and 6 at a slightly faster rate than the "fast" genetic variant.

Preliminary experiments with small sample sizes indicate that during Phase 4, bands 5 and 6 also follow a cycle of activity similar to Phase 2 (Fig. 2).

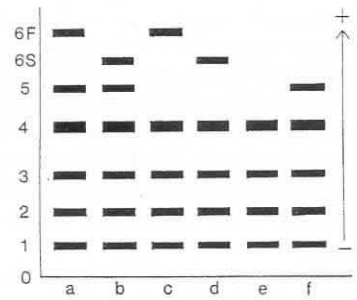


Fig. 1. Esterases in *Folsomia candida*. (a) Control-Fast type (Phase 1 or 3); (b) Control-Slow type (Phase 1 or 3); (c) Fast type (24—30 hours into Phase 2); (d) Slow type (24—30 hours into Phase 2); (e) Either fast or slow type (6—18 hours into Phase 2); (f) Either fast or slow type (0—6 hours into Phase 2). Time periods indicate when these esterase patterns are likely to be seen during the molt cycle.

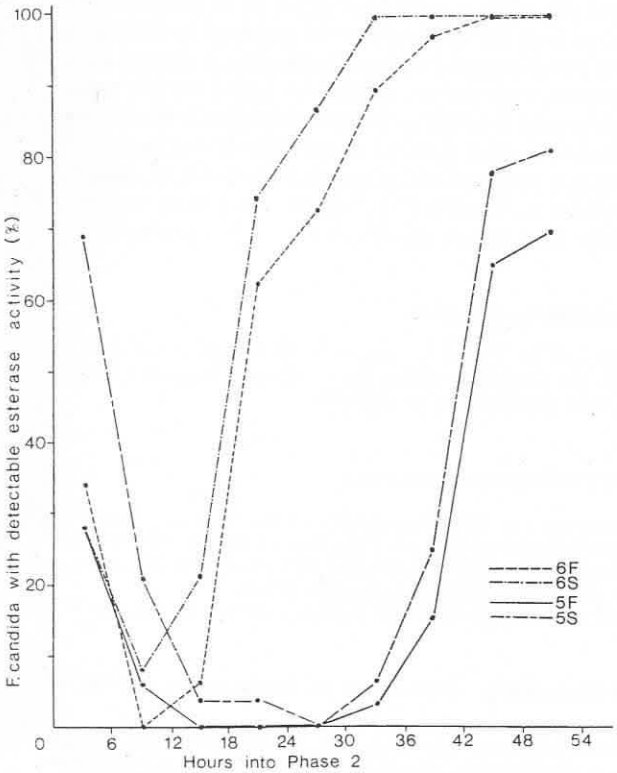


Fig. 2. Cycle of detectable esterase activity in *Folsomia candida*. Esterase bands 5 and 6 of both genetic variants (Fast 5F, 6F; Slow 5S, 6S) are shown. Each data point represents 32 individual *F. candida* whole body homogenates.

## 4. Discussion

In many isozyme studies, the physiological state of the test organism appears to be unknown. As a result description of isozyme variation for use in systematics or population studies often contain physiological as well as genetic variation. Physiological variation may be quite important in organisms, such as Collembola, since they continue to molt throughout their entire lifetime. Metabolic changes in those structural proteins and enzymes involved either directly or indirectly in the molt cycle should not be unexpected.

The present paper deals with one example of physiological variation in isozyme titer or activity. Two esterase isozymes in *Folsomia candida* show differences in staining intensity correlated to the molt cycle. If the *F. candida* population used in the present study had been sampled randomly with respect to time within the molt cycle (physiological state), each of the six esterase zymograms (Fig. 1a—f) would have appeared. The resulting data would have indicated six different genetic variants, with bands 5 and 6 occuring less frequently than the stable bands 1—4. Heritable variation within this population would have been overestimated. It was only by careful attention to the physiological state of the *F. candida* population that the cycling nature of the esterase enzymes was revealed.

In *F. candida*, HART & ALLAMONG (1979) showed a positive correlation between body size (indicative of age) and the number of esterase bands present in the enzyme homogenates. Eggs and first instar animals had only two esterase bands, but the number of enzyme bands increased with size so that in the adult population eight bands were present. Indiscriminate mixing of developmental stages or ages during isozyme studies may represent another source of variation.

Given the evidence presented here and the work reported by HART & ALLAMONG (1979) and ASHER & SNIDER (1975), variation in isozyme electrophoretic patterns in Collembola may be based on genetic, physiological or developmental factors. Estimates of genetic variation in *F. candida*, either within or between populations, should be based on the proper choice of a developmental and physiological time interval in which no changes in the isozyme titer or activity can be detected. Furthermore, since molting frequencies in Collembola are highly species specific (HALE 1965), the target enzymes within the species should be analyzed for physiological variation before the species is examined as a whole. Only then will it be possible to compare isozyme data between and within species in the most meaningful and efficient way.

## 5. Acknowledgements

The author wishes to thank R. M. SNIDER and Dr. JAMES H. ASHER for their advice and criticism throughout this work, and Dr. RALPH PAX for his review of this paper. Support from the Zoology Department, Michigan State University, is also gratefully acknowledged.

## 6. Summary · Zusammenfassung

Starch gel electrophoresis of esterase isozymes of *Folsomia candida* (WILLEM) revealed enzyme variation correlated with molt cycle stages (physiological variation). Two esterase enzyme bands disappeared during the fasting period preceding the molt, but the precise timing, rate of disappearance and rate of reappearance were different for each of the two enzyme bands.

The possible interference of physiological variation with the genetic variation within the Collembola population is discussed.

**Esterasen in *Folsomia candida* (Collembola: Isotomidae). Änderungen des Isozym-Titers während des Häutungszyklus**

Stärke-Gel-Elektrophoresen von Esterase-Isozymen von *Folsomia candida* (WILLEM) deckten Änderungen des Enzymspiegels auf, die mit Stadien des Häutungszyklus korreliert sind (physiologische Variation). Zwei Esterase-Enzymbänder verschwinden während der Fastenperiode, die der Häutung vorangeht; der präzise Zeitablauf der Raten des Verschwindens und des Wiederauftretens war für jedes der beiden Enzymbänder verschieden. Die mögliche Interferenz der physiologischen Variation mit der genetischen Variation innerhalb der (untersuchten) Collembolenpopulation wird diskutiert.

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